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EFFECT OF S-ADENOSYLMETHIONINE (SAME) IN GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

Objectives: *Gentamicin nephrotoxicity is a common clinical situation in patients who receive it for a longer time period. The objective of the present study is to assess the effect of SAMe (S –Adenosyl Methionine) - a precursor of Glutathione (endogenous antioxidant) in gentamicin induced nephrotoxicity in rats, by measuring serum urea, serum creatinine and histopathological analysis.* ***Methods:*** *Three groups (n=6) of Sprague Dawley rats weighing around 200-250 grams were used in the study. First group received normal saline (0.9%) 1 ml intramuscular (i.m), for 5 days, second group received 80m/kg of gentamicin i.m, for 5 days and the last group received 80mg/kg of gentamicin i.m and SAMe 5mg/kg intraperitoneal for 5 days. 24 hrs after the last injection, the rats were anaesthetized and blood was collected by cardiac puncture and sent for estimating serum urea and creatinine. The kidneys were dissected out and sent for histopathological analysis and were graded for percentage change of tubular necrosis. The results of the study were based on Kruskal Wallis test for among the groups and Mann Whitney U test for between the two groups. The measure of central tendency used for analysis was median.* ***Results:*** *The results of the study has shown that the group which had used SAMe + gentamicin had a slightly greater urea and creatinine, compared to gentamicin alone (p=0.630, 0.626) and saline alone group (p=0.335, 0.217), even though it was not statistically significant. Even among the groups, there was no statistical significance (p=0.279, 0.335).Similarly in the histopathological analysis, there was equivocal findings with respect to proximal tubular necrosis in both gentamicin alone and SAMe + gentamicin group.* ***Conclusions****: The results from the present study indicate that SAMe does not improve the nephrotoxicity caused by gentamicin, when assessed with serum urea, serum creatinine and histopathology.*

KEY WORDS

Gentamicin, SAMe, Nephrotoxicity.

INTRODUCTION

Gentamicin is one of the most commonly used antibiotics for the treatment of gram negative infections. It belongs to the group of antibiotics called aminoglycosides. When it is used for these infections for a week or two, it does not lead to any adverse effects, but prolonged use can lead to nephrotoxicity and ototoxicity.

Out of these two adverse effects, the incidence of nephrotoxicity manifests as increasing serum urea and

creatinine, and these variables guide the course of therapy to prevent further nephrotoxicity. The vulnerable population to this adverse effect is the elderly, subjects with liver disease, diabetes mellitus, and patients with septic shock,[1]but it can also affect other age groups if given for a long time.[2]

Numerous experiments have evaluated the nephrotoxicity caused by gentamicin in both animals and human beings and various theories, have been put forward to explain it. Of all these theories, the most plausible ones have been Phospholipidosis theory [3, 4, 5] and the Oxidative stress theory. [6]

Based on these theories, numerous drugs have been discovered and have been tried experimentally, some with success and others not. This study aims to evaluate the effect of an already existing drug called S-Adenosyl Methionine or SAMe, which is known for its antioxidant properties, by acting as a precursor of Glutathione, the major cellular antioxidant [7] and hence is used in various indications where oxidative stress plays a role. [8, 9] Apart from its role as an antioxidant, it is also known to increase the synthesis of Polyamines, [10]which are known for their cellular regenerative actions.

MATERIALS AND METHODS

Animals

Adult Sprague Dawley rats of either sex weighing 200-250 grams were used in the study. All rats were housed under habitual conditions in a temperature controlled room at 22 degree Celsius, with a 12 hour light/dark cycle with free access to Water and standard laboratory chow. This animal study was approved by Institutional Animal Ethics Committee [6942-30/9/2009]. The study was done as per the guidelines set by CPCSEA, India.

Drugs

The drugs which were used in the study were normal saline (0.9%), gentamicin and S Adenosyl Methionine. Gentamicin and saline were obtained from the Hospital Pharmacy of the Christian Medical College and Hospital, Vellore, India. The drug S -Adenosyl Methionine (SAM) was a gift from Orchid Pharmaceuticals, Chennai, India.

Study Design

There were 3 groups in the study and 6 animals in each group (n=6).The first group (control) received 1ml of sterile saline I.M (intra muscular) for 5 days. The second group received 80mg/kg of Gentamicin I.M for 5 days, and the third group received both gentamicin 80mg/kg I.M and SAMe 5mg/kg I.P (intraperitoneal) for 5 days. Twenty four hours after the last injection, all rats were anaesthetized with Ketamine and sacrificed by cardiac puncture and blood was sent for estimation of Serum urea and serum creatinine. The renal tissues were dissected out and sent for histopathological analysis.

Biochemical methods

Urea was measured enzymatically using the kit, Autozyme, Accurex biochemical Pvt. Limited. Creatinine was measured in an in house method based on Jaffe's method.[11]Assays were carried out in Roche Modular P 800 automated chemistry analyzer.

Histopathology processing and staining

The nephrectomies were bisected and sent to the laboratory in 10% buffered formalin (10 times the volume of tissue). The specimen was fixed overnight following which 0.2cm thin sections were taken. These sections were placed in the tissue cassettes. For further processing these cassettes were placed in an automated tissue processor and dehydrated with increasing grades of alcohol followed by immersion in

clearing agent and then impregnation with paraffin wax. The tissue was then embedded into paraffin blocks. From the blocks, 4 micron tissue sections were cut with the help of a microtome, floated in the water bath to prevent wrinkles and folds and then placed on the slides precoated with an adhesive (egg albumin and glycerine). The slides were then incubated at 60º for 1 hour. The slides were then deparaffinized with zylene followed by rehydration with decreasing concentrations of alcohol and finally with water. The sections were then stained with haematoxylin (obtained from heart wood of the tree *Haematoxyloncampechianum*) for nuclei and eosin (acid xanthene or phthalein dye) for cytoplasm. Then following dehydration and clearing, the slides were mounted with DPX (Dystrine Phthalate Xylene) solution.

Analysis of histopathological sections:

A light microscopic semiquantitative analysis of the kidney sections was performed using the technique of Houghton et.al.[12] The changes seen were limited to the tubulointerstitial areas and were graded as follows:

0. Normal

1. Areas of focal granulovacuolar epithelial cell degeneration with < 1% of total tubule population showing epithelial cell desquamation;

2. Tubular epithelial necrosis and desquamation involving <50% of cortical tubules.

3. Greater than 50% of proximal tubules showing desquamation (uninvolved tubules easily found).

Statistical Methods:

All the test results were based on median values. For comparison of two groups at a time Mann-Whitney U test was used. For the comparison among groups, the Kruskal Wallis test was used. A P value of <.05 was considered statistically significant.

RESULTS

The results of the present study were based on the Kruskal Wallis significant test for among the groups and Mann Whitney U test for between 2 groups. The measure of central tendency used for the analysis was median.

Effect of treatment on urea and creatinine:

The result for both urea and creatinine in the SAM + Gentamicin interventional group shows a marginal increase, compared to the Gentamicin alone group (**Table 1**). Even then we could not say that the intervention viz. SAMe increases the nephrotoxicity because of its statistical insignificance.

Semi quantitative analysis of renal histology:

The histological changes were graded as described in methods and the results are expressed in **Table 2**.

When compared to control group (**Figure 1**), in rats treated with gentamicin (figure 2) the tubular necrosis varied from null (Grade 0) to more than 50% (Grade1, 2).Even in the interventional group namely SAMe + gentamicin (figure 3) the results were equivocal with tubular necrosis varying from null (Grade 0) to more than 50% (Grade 1, 2). Hence there is not any significant change in histopathology to prove that either of the groups is better than the other. The results from the above analysis have shown that giving SAMe to rats with gentamicin induced nephrotoxicity actually does not improve the nephrotoxicity.

Outcome	n	Median	IQR	P value
Urea levels				
Saline	6	**43.00**	**3.25**	
Gentamicin	6	**48.00**	**14.25**	**0.279**
SAMe+Gentamicin	6	**52.50**	**24.00**	
Creatinine levels				
Saline	6	**0.650**	**0.10**	
Gentamicin	6	**0.750**	**0.40**	**0.335**
SAMe+Gentamicin	6	**0.900**	**0.55**	

Table 1: Urea and creatinine levels in the SAMe + Gentamicin interventional group compared to the Gentamicin alone group.

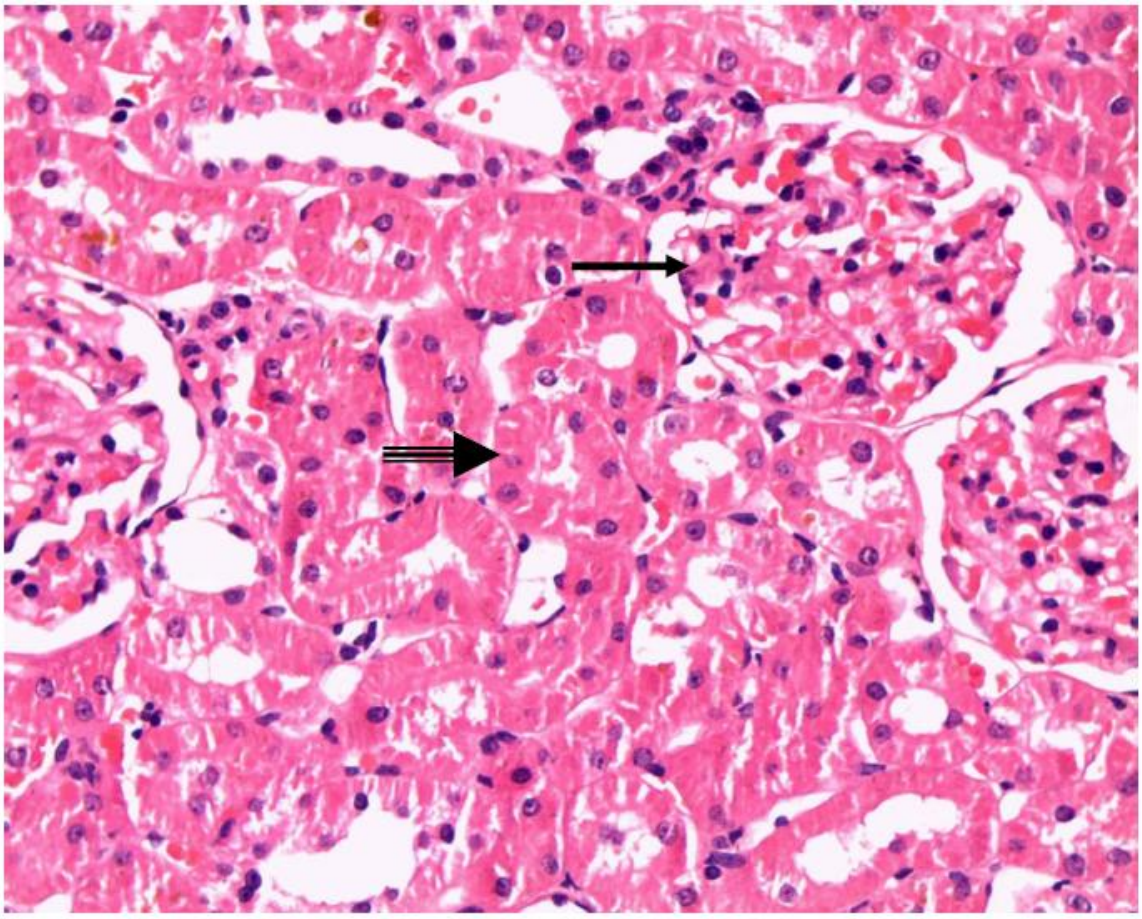
Group	Grade 0	Grade 1	Grade 2	Grade 3
Saline	6			
Gentamicin	3	1	2	-
SAMe +Gentamicin	2	2	2	-

Semi quantitative analysis of renal histology*

Table 2: *Histologic grading as follows: 0 = normal; 1 = Areas of focal granulovacuolar

Epithelial cell degeneration with < 1% of total tubulepopulationshowingepithelialcell desquamation; 2 = Tubular epithelialnecrosis and desquamation involving < 50% of cortical tubules;3 = Greater than50% of proximal tubules showing desquamation (Uninvolved tubules easily found);

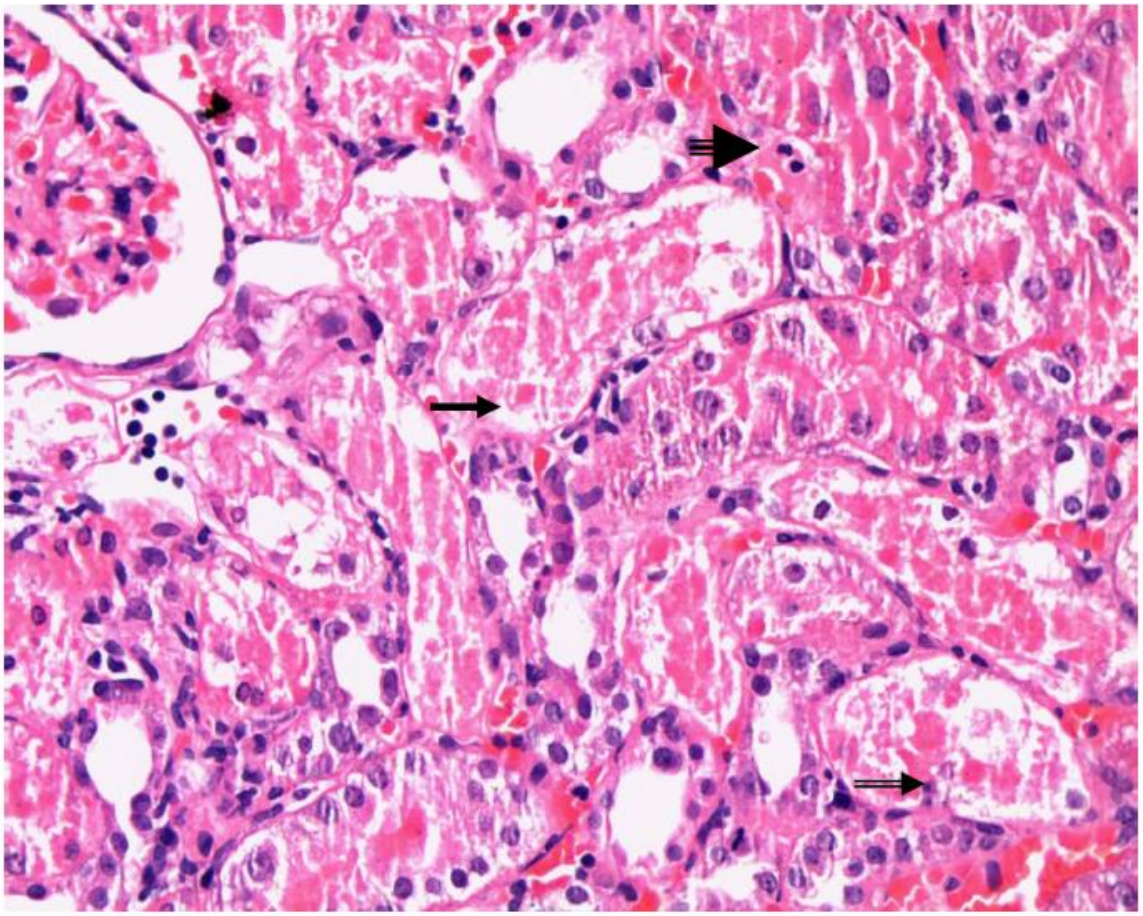
Figure 1: Histopathological section of kidney (normal saline)



Normal kidney glomerulus

Proximal convolutes tubules; Note small nuclei with almost inconspicuous nucleoli

Figure 2: Histopathological section of kidney (gentamicin group,showing tubular necrosis).

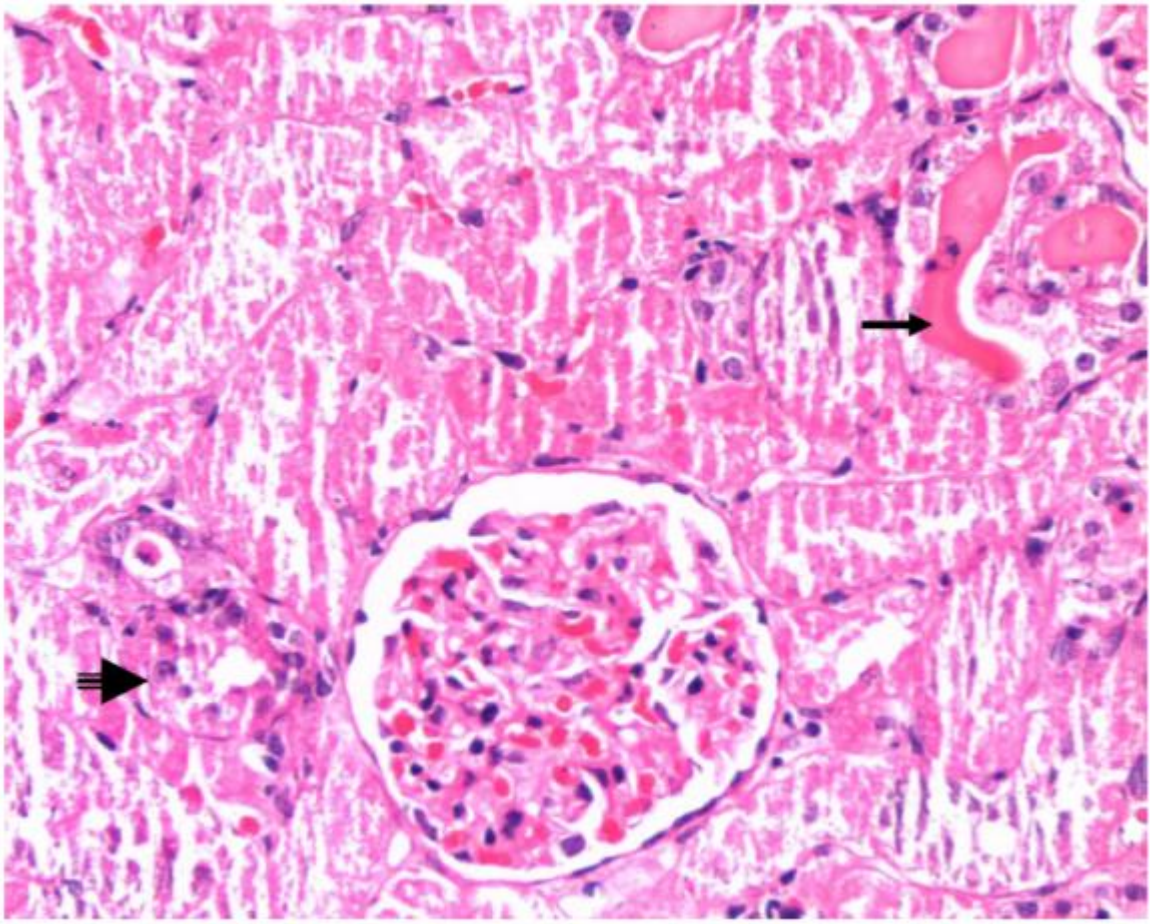


Loss of epithelial lining

Nuclear debris

Inflammatory infiltrate

Figure 3: Histopathological section of kidney (SAMe +gentamicin group,showing Tubular necrosis)



Almost complete tubular necrosis with occasional viable tubule

Hyaline cast

DISCUSSION

Nephrotoxicity induced by gentamicin is a common finding in patients who receive it for a longer course of time to combat gram negative infections. Out of the various theories which explain the nephrotoxicity of gentamicin the impressive ones are the theory of phospholipidosis and oxidative stress. Although there are studies which have shown a positive response with antioxidants like Vitamin E, selenium [13] and even melatonin [14] the search for a newer drug with a favorable action is always encouraged, to show if there is any difference. SAMe is an important endogenous compound found in the body and abnormalities in SAMe metabolism have been demonstrated in many disorders of the body like hepatic and central nervous system disorders. SAMe has been shown to have therapeutic value in disorders such as hepatic disorders,[9]major depressive disorder,[15] Alzheimer's disease,[16] fibromyalgia,[7] osteoarthritis[18]and paracetamol overdose.[19] For all these conditions the beneficial action of SAMe is proposed to be due to its antioxidant effect.

In the present study, we have tested the drug SAMe which is a precursor of the most powerful endogenous

antioxidant i.e. glutathione, in the nephrotoxicity induced by gentamicin. It was reasonable to use this drug, because the results from a previous study has shown that SAMe has a role in suppressing renal oxidative stress [20] and oxidative stress has also been proved to play a role in gentamicin induced nephrotoxicity.[6]Moreover SAMe also is involved endogenously in aminopropylation, which helps in the synthesis of polyamines.[10] Since polyamines are a group of compounds which help in cellular regeneration, we could anticipate a beneficial role for SAMe in cellular regeneration, when the drug gentamicin had caused necrosis of the proximal convoluted tubular epithelial cells, after its administration in toxic doses.

Here we measured the blood urea and serum creatinine for 3 groups of rats viz, Saline, Gentamicin and SAMe + gentamicin, and also assessed the histopathology of the renal sections of the respective groups.

The results of this study have shown that the group which had used SAMe + gentamicin had a slightly greater urea and creatinine, compared to the gentamicin alone (p=0.630, 0.626) and saline alone group (p=0.335, 0.217), even though it was not statistical significant.Even among the groups there was no significance, when assessed by the KruskalWallis test (p=0.279, 0.335). Similarly in histopathological sections there was equivocal finding with respect to proximal tubular necrosis in both the gentamicin alone and SAMe + gentamicin group(see table 1).But the marginal increase in the urea and creatinine in SAMe+ gentamicin group ,compared to gentamicin alone group does have little clinical significance,because in a recent study where SAMe was used for its antioxidant role against the nephrotoxicity caused by Cisplatin (oxidative stress has been one of the theories to explain Cisplatin nephrotoxicity),there was a significant rise in blood urea nitrogen and creatinine in the Cisplatin+SAMe group compared to Cisplatin alone [21] proving that SAMe actually caused a significant increase in renal dysfunction.

CONCLUSION

The results from the present study indicate that even though SAMe is an attractive option theoretically as an antioxidant to treat gentamicin induced nephrotoxicity, in reality it does not improve the nephrotoxicity caused by gentamicin, when assessed with serum urea, serum creatinine and histopathology.

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